DXC 800 (CSAL) CYCLOSPORINE

☒ St. Joseph Medical Center, Tacoma, WA	☐ St. Anthony Hospital Gig Harbor, WA	☐ Harrison Medical Center, Bremerton, WA
☐ St. Francis Hospital, Federal Way, WA	☐ St. Elizabeth Hospital Enumclaw, WA	☐ Harrison Medical Center, Silverdale, WA
☐ St. Clare Hospital Lakewood, WA	☐ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instructions for the quantitative determination cyclosporine in whole blood using the DXC 800.

PRINCIPLE

Cyclosporine reagent, when used in conjunction with UniCel® DxC 800 System(s), is intended for the quantitative determination of cyclosporine concentration in human whole blood.

BACKGROUND

Clinical Significance

Cyclosporine is a hydrophobic cyclic undecapeptide of fungal origin with immunosuppressive properties. Although its mechanism of action is still under investigation, cyclosporine appears to affect the metabolism of T-helper lymphocytes and T-suppressor lymphocytes, resulting in an impairment of the immune system. The immunosuppressive properties of cyclosporine make it a very effective drug for the treatment of certain autoimmune diseases and reducing the incidence of tissue rejection following organ transplantation. Cyclosporine therapy has optimal safety and efficacy over a narrow range of concentrations and may lead to a number of adverse effects. The most critical adverse effects are organ rejection from inadequate dosing, or nephrotoxicity and hepatotoxicity, which become more probable as the drug concentration is increased. Cyclosporine is administered either orally or intravenously. Since absorption and hepatic metabolism of the drug are highly variable from patient to patient, there is a poor correlation of blood levels with the administered dose. Factors affecting cyclosporine concentrations in the blood include the nature of the transplant, the age and general health of the patient, and the co-administration of drugs such as carbamazepine, phenytoin, phenobarbital, erythromycin, rifampin, cimetidine and ketoconazole. It is essential to monitor cyclosporine in organ transplantation to achieve optimal immunosuppressive effects in patients.

The measurement of cyclosporine concentrations in whole blood in conjunction with other laboratory data and clinical evaluation is the best approach to optimize immunosuppression and minimize adverse side effects for recipients of organ transplants.

Methodology

The CEDIA Cyclosporine PLUS assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system. The assay is based on the bacterial enzyme ß-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzymes that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment of ß-galactosidase for antibody binding site. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzymes. If analyte is not present in the sample, antibody binds

to analyte conjugated on the inactive fragment, inhibiting the reassociation of inactive ß-galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.

RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
J-F-CH-0826	DXC 800 Calibrators
J-F-CH-1940	DXC 800 (AMR) Analytical Measurement Range

SPECIMEN

Type of Specimen

The sample required for this assay is 100 uL of whole blood drawn in an EDTA vacutainer tube.

Specimen Storage and Stability

The specimens should be refrigerated at 2-8 C if the assay is not to be performed that day. Refrigerated specimens are stable for at least one week.

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

REAGENTS

User-defined CEDIA Cyclosporine PLUS Assay kit

1 EA Reconstitution Buffer: Contains MOPS [3-(N-morpholino) propanesulfonic acid buffer], 0.50 µg/mL mouse monoclonal anti-cyclosporine antibodies, stabilizer, and preservative, 1 x 41 mL.
1a EA Reagent: Contains 0.171 g/L Enzyme Acceptor (microbial), buffer salts, and preservative, 1 x 41 mL.
2 ED Reconstitution Buffer: Contains MES [2-(N-morpholino) ethanesulfonic acid buffer], detergent, and preservative, 1 x 19 mL.
2a ED Reagent: Contains 52 µg/L Enzyme Donor(microbial) conjugated to cyclosporine, 2.73 g/L chlorophenol red-ß-D-galactopyranoside, stabilizers, and preservative, 1 x 19 mL.
3 Lysing Reagent: Contains buffer salts, detergents, and preservative, 1 x 98 mL.
4 Low Range A Calibrator: Contains 0.45 g BSA and 0.063 µg Cyclosporine A.
5 Low Range B Calibrator: Contains 0.45 g BSA and 1.125 µg Cyclosporine A.

Reagent Preparation

<u>R1 (Enzyme Acceptor) reagent preparation</u>

1. Use **Cold** reagent

2. Connect bottle 1a (EA Reagent) to the 70mL
   Bottle 1 (EA Buffer) by using the enclosed adapter.

3. Mix by gentle in-version (avoid foaming) ensuring that all the lyophilized material is transferred to the 70mL bottle. Cap and allow to stand 5 min.

4. Mix again and transfer contents to Reagent compartment A in a user-defined cartridge.

R2(Enzyme Donor)reagent preparation

1. Use **Cold** reagent

2. Connect bottle 2a (ED Reagent) to Bottle 2 by using the enclosed adapter

3. Mix as above, transferring contents to Bottle 2. Cap and allow to stand 5 min.

4. Mix again and transfer contents to reagent compartment B in a user-defined cartridge.

5. Label the cartridge as “CSAL”, include the lot number and expiration date (60days)

Lysing Reagent

Mix by gentle inversion 2-3 times before use.
Stable for 60 days at 2-30 C.

Acceptable Reagent Performance

The acceptability of a reagent is determined by ensuring that quality control results are within your facility`s acceptance criteria.

CALIBRATION

Calibrator Required

CEDIA Cyclosporine PLUS Assay Kit

Low Range Cal A
Low Range Cal B
These are stable for 60 days at 2-8 C.

These calibrators are to be pre-treated in the same manner as the controls and patients.
The values are given in the package insert for each kit, are lot specific and need to be updated in the analyzer as necessary. Calibration is required every 7 days or when a new reagent cartridge is loaded. Run calibration as specified tin the DXC 800 Operating Manual.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls

BioRad Lyphochek Whole Blood Controls Levels 1 & 2

Reconstitute controls with 2.0 mL deionized water
Allow to stand 60 min, swirling occasionally.
Stable for 14 at 2-8 C after reconstitution

STEPS

1. Allow calibrators, controls and patient samples to come to room temp on rocker.

2. Label appropriate tubes for each calibrator, control and sample to be tested and place in a suitable rack.

3. Accurately pipet 100uL of the sample to be assayed into its corresponding tube.

4. Using an Eppendorf pipette (setting at 4) accurately dispense 400uL of Lysing Reagent into each sample tube.

5. After pipetting all the samples, cap each tube securely and mix each on a vortex mixer for 2-5 seconds to ensure thorough mixing.

6. Run the assay promptly after mixing.

PERFORMANCE CHARACTERISTICS

Reference Range

Type	Range
Therapeutic Range Renal Transplants	100-200 ng/mL
Therapeutic Range all other Transplants	100-300 ng/mL
Toxic Therapeutic Response	>400 ng/mL Report **CRITICAL**
Ineffective Therapeutic Response	<50 ng/mL

Reporting results outside of analytical range

Lower limit of detection	25 ng/mL	Results below 25; Report as <25 ng/mL
Upper limit of linearity	450 ng/mL	Follow dilution protocol below. The maximum allowable dilution is X2. Results >900 are reported as >900 ng/mL.

Results >450 need to be diluted. Dilute by adding 50uL of patient sample and 50uL of cyclosporine free whole blood to an appropriately labeled tube. (Run an extra lavender tube, ie. CBC, for cyclosporine to find one that is negative). Add 400uL of the Lysing Reagent and vortex. Reanalyze and multiply the result by the dilution factor of 2.

LIMITATIONS

None identified.

REFERENCES

CEDIA Cyclosporine PLUS Assay Instructions, Microgenics

DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Formatting, added related documents, removed section about Cerner ordering and resulting and all other references to Cerner, added max dilution, added clinical significance and methodology, reagents from manufacturer procedure. Allow to stand 60 min, swirling occasionally.			
☐ *No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.*			
Committee Approval Date	☐ Date: ☒ N/A – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 3/17/16